Urea metabolism in beef steers fed tall fescue, orchardgrass, or gamagrass hays[1,2]

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ABSTRACT: Two experiments were conducted to assess effects of endophyte treatments (Exp. 1), forage species (Exp. 2), and supplementation (Exp. 2) on urea production, excretion, and recycling in beef steers. Infusion of ^{15,15}N-urea and enrichment of urea in urine samples were used to calculate urea-N entry and recycling to the gut. Acceptably stable enrichment of ^{15}N-urea in urine was obtained after 50 h of intrajugular infusion of ^{15,15}N-urea, indicating that valid data on urea metabolism can be obtained from steers fed forages twice daily. After adjustment by covariance for differences in N intake among treatments in Exp. 1, steers fed endophyte-infected tall fescue had less ($P < 0.10$) urea-N entry, recycling to the gut, and return of recycled urea-N to the ornithine cycle than those fed endophyte-free or novel endophyte-infected tall fescue. However, urea-N urinary excretion or return to the gut was similar among endophyte treatments when expressed as a proportion of urea-N entry. Urea-N entry and return to the gut in Exp. 2 was similar in steers fed gamagrass or orchardgrass hay after adjustment by covariance for differences in N intake. Less ($P < 0.01$) urinary excretion, expressed as grams per day or as a proportion of urea-N entry, with gamagrass than with orchardgrass was associated with faster in vitro NDF-N digestion with gamagrass. Supplementation of gamagrass or orchardgrass with 1.76 kg/d of readily fermentable fiber and starch decreased urea entry ($P < 0.06$) and urinary excretion of urea ($P < 0.01$). Interactions between hay source and supplement reflected a greater response to supplementation for steers fed orchardgrass than for those fed gamagrass. After adjustment for differences among treatments in N supply, results of both experiments support the concept of improved N use in response to increased carbohydrate fermentability in the rumen, due either to inherent differences in forage fiber or to supplementation with readily fermentable carbohydrate (starch or fiber). Closer coordination of ruminal fermentation of carbohydrate and N sources provided greater and more efficient capture of dietary N as tissue protein in forage-fed steers.

Key words: fescue, gamagrass, orchardgrass, steer, urea metabolism

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INTRODUCTION

Extensive hydrolysis of nitrogenous compounds in the rumen creates a large supply of ammonia, much of which is absorbed into the bloodstream of the ruminant (Reynolds and Kristensen, 2008). Forages are of particular interest because they are high in readily degradable NPN (NRC, 2000; Archibeque et al., 2001, 2002; Magee et al., 2005; Huntington et al., 2007; Huntington and Burns, 2008). Because of the close link between ammonia supply and urea metabolism, ^{15,15}N-urea is a useful way to evaluate N use in ruminants by quantifying urea production, urinary excretion, and return to the gut (Lobley et al., 2000; El-Kadi et al., 2006; Kiran and Mutsvangwa, 2007; Sunny et al., 2007; Gozho et al., 2008). Our interest has focused on forage-fed beef steers and changes in their urea metabolism in response to forage species, forage composition, or supplements of energy or AA (Archibeque et al., 2001, 2002; Huntington and Burns, 2008). Synchronization of energy and protein in the rumen may improve capture of dietary N as microbial N, thereby reducing ammonia absorption, urea production, and loss of N through urinary excretion (Reynolds and Kristensen, 2008). We are not aware of published data to support an acceptable

[1]Use of trade names in this publication does not imply endorsement either by the North Carolina ARS or USDA-ARS or criticism of similar products not mentioned.

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time frame for infusion of ^{15,15}N-urea before collection of urine samples for enrichment in animals fed twice daily; studies have established a 48-h minimal time for sheep fed small, regular meals (Lobley et al., 2000; El-Kadi et al., 2006), and others have used at least 48 h in steers fed twice daily (Archibeque et al., 2001, 2002). The main objectives of the experiments in these studies were 1) to assess the roles of forage species, endophyte treatment, and composition on urea kinetics in beef steers; 2) to identify interaction between supplementation with readily degradable carbohydrate and forage species; and 3) to verify that 48 h of infusion is a suitable minimal time frame for ^{15,14}N urea enrichment in urine of beef steers fed twice daily.

MATERIALS AND METHODS

Forage composition, intake, digestion, N retention, and blood urea-N data from these studies have been published (Magee et al., 2005; Matthews et al., 2005). The North Carolina State University Institutional Animal Care and Use Committee approved the use of steers in these studies.

Exp. 1

The experimental design was a replicated 3 × 3 Latin square. Details on diets, animals, and procedures have been published (Matthews et al., 2005). Endophyte-infected, Jesup tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh., **E+**], endophyte-free Jesup tall fescue (**E−**), and nontoxic endophyte-infected Jesup tall fescue (**NE**; MaxQ, Pennington Seed Inc., Madison, GA) were harvested as hay and stored indoors until feeding (Table 1). Each hay (treatment) was sliced with a 5600 Van Dale Bale Processor (J. Starr Industries, Ft. Atkinson, WI) before feeding. Eight Polled Hereford steers (average initial BW 240 ± 9 kg) were used, with an extra steer allotted to each square. Before the experiment, steers were weighed and treated with Cydectin Pour-on (Fort Dodge Animal Health, Overland Park, KS) for elimination of internal parasites. Steers were blocked by BW into 2 groups (squares) and housed in individual tie stalls (115 × 178 cm) with individual feeders and water cups. Group 1 preceded group 2 through the protocol by 2 wk. Steers were fed for ad libitum intake for 14 d, and then hay offered was restricted for 14 d to an amount equal to the lowest individual ad libitum intake (% of BW) for that period within square. Room temperatures were controlled and ranged from 21.4 to 27.3°C during the experiment. Orts, feces, and urine were collected during the last 5 d of restricted intake. Steers were fed at 0900 and 1600 h daily. At 0900 h, each steer received 57 g/d of a mineral supplement that consisted of a 1:2 mix of dried molasses and a commercial mineral mix (Southern States Cooperative, Richmond, VA; 16.5% Ca, 7.0% P, 24.5% NaCl, 3.5% Mg, 2.0% S, 1.0% K, 70 mg/kg of I, 1,500 mg/kg of Cu, 32 mg/kg of Co, 52 mg/kg of Se, 3,200 mg/kg of Zn, 3,000 mg/kg of Mn, 118 IU/g of vitamin A, 9.1 IU/g of vitamin D, 91 IU/kg of vitamin E). The mixture was offered before the 0900 h feeding.

Table 1. Chemical composition (g/kg of DM) of endophyte-infected (E+), endophyte-free (E−), and nontoxic endophyte (NE) tall fescue in Exp. 1[1]

	Tall fescue			
Item	E+	E−	NE	SEM
CP	108[a]	118[b]	116[b]	0.9
NDF	599[a]	585[b]	586[b]	3.6
ADF	294[a]	284[b]	283[b]	1.8

[a,b]Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]n = 6 for each hay. Data from Matthews et al. (2005).

Urine, feces, and orts were collected at 0800 h each morning as described by Matthews et al. (2005). Collections began on a Sunday and lasted until the following Friday. Isotope infusion began for 4 steers on Sunday, and isotope infusion for the remaining 4 steers began on Wednesday. Before infusion of the isotope, urine samples were collected for each steer. Steers were infused intrajugularly with ^{15,15}N-urea (CA #2067–80–3, Cambridge Isotope Laboratories Inc., Andover, MA) prepared in a sterile 0.9% NaCl saline solution. Isotope infusion rates were maintained at 85 mL/h for 56 h using a peristaltic pump (model 1000, International Medical Systems Inc., Huntington, NY), and the ^{15,15}N-urea infusion rate was 0.272 mmol of urea-N/h. Urine was collected 52, 54, and 56 h after the start of infusion, and a subsample was stored frozen until it was analyzed for urea concentration and enrichment with ^{15,15}N- and ^{15,14}N-urea.

Exp. 2

The experimental design was a split-plot with hay as the whole plot factor and supplement as the split-plot factor balanced in time across hays. The experiment began in June and ended in August, 2002. Treatments were Iuka gamagrass [*Tripsacum dactyloides* (L.) L.] hay or Potomac orchardgrass (*Dactylis glomerata* L.) hay fed with or without a soybean hull/corn supplement mix (Table 2). Molasses was added to the supplement to enhance palatability and consistent consumption by the steers.

Eight Hereford steers (initial BW was 276 ± 31 kg) were trained to be led by halter and accustomed to close human contact, passage through the animal handling facilities, and familiarization with equipment necessary for blood, urine, and fecal collections.

Steers were implanted with Synovex-S (200 mg of progesterone and 20 mg of estradiol benzoate, Fort Dodge Animal Health, Fort Dodge, IA) in the middle of the left ear between the skin and cartilage, assigned by BW into a heavy group and a light group, and then randomly assigned orchardgrass or gamagrass hay (4

Table 2. Composition of hays and supplement in Exp. 2

Item	Supplement[1,2]	Gamagrass[3]	Orchardgrass[3]	SEM[4]
DM, g/kg	870	919	918	0.5
CP, g/kg of DM[a]	113	84	119	1.4
OM, g/kg of DM[a]	961	950	919	0.4
NDF, g/kg of DM[b]	342	706	719	4.7
ADF, g/kg of DM[a]	229	362	379	3.2

[a]Hays differ ($P < 0.01$).
[b]Hays differ ($P < 0.10$).
[1]Contained 50 g of molasses per kg of supplement.
[2]n = 2 for supplement.
[3]n = 7 for each hay.
[4]SEM of hays.

steers per hay). Steers fed gamagrass weighed 289 ± 34 kg and steers fed orchardgrass weighed 262 ± 25 kg at the beginning of the experiment. During period 1 of the crossover, steers were randomly assigned to receive either control (no supplement), or a 50:50 mixture of corn grain and soy hulls. Four heavier steers shared a pen with individual Calan gates (American Calan Inc., Northwood, NH) and the 4 lighter steers shared a similar adjacent pen. The pens were under a roof. Steers were adapted to the Calan gate feeding system for approximately 1 wk and fed wheat hay for ad libitum intake during this phase. Once the steers were adapted to the Calan gates, a 21-d ad libitum intake phase began. Intake during the last 8 d was used to calculate ad libitum DMI for each steer. Following the ad libitum phase, steers were housed indoors in metabolism crates with a 7-d adjustment phase followed by a 5-d collection phase. Natural daylight determined the light and dark time in the barn, which was approximately 14.5 h of light daily during the experiment. Steers had ad libitum access to water and a trace-mineralized salt block throughout the study. While steers were housed in metabolism crates, barn temperatures were monitored and box fans were used to circulate air. Barn doors remained open to aid in airflow. Average barn temperature was 27°C during period 1 and 23°C during period 2.

Hay was initially fed at 2% of BW daily and subsequently offered at 110% of the intake of the previous day during the ad libitum phase. Orts were removed and weighed daily. At DMI equal to 2% of BW daily, unsupplemented diets contained CP and TDN to support maintenance and 0.454 kg of ADG (NRC, 2000). Hay samples were taken periodically throughout the study before passing the bales through the 5600 Van Dale Bale Processor. These intact samples were composited and a subsample was later hand-separated into leaf, stem, heads, and other material. The heads were severed, in the case of orchardgrass, directly below the bottom ray of the panicle with the remaining peduncle left with the stem fraction. In the case of gamagrass, the cut was made directly below the bottom ovule with the seed stalk combined with the stem fraction. The supplement was fed at a constant rate of 1.76 kg of DM/d, which was 7.5 g/kg of the average initial BW of the steers. Supplement was fed at 0830 and 1600 h in 2 equal portions. Hay was fed at 0900 and 1630 h in 2 equal portions.

During the balance trial, hay was fed at 90% of the previous 5-d average of the ad libitum intake of a given steer. Supplement was fed at 90% of 1.76 kg of DM/d as well. Before the balance trial collection, all crates were thoroughly scrubbed and washed. Plastic tarps were placed directly behind the crates for fecal collection, and urine collection containers were placed under the crates.

Hay was sampled and composited daily. Grab samples of the supplement were also taken daily. The urine pans contained a daily allotment of water and 6 *M* HCl for acidification to ensure that the collected urine was maintained at a pH <4. This was verified using pH-sensitive paper before collection of the aliquot. Feces and urine were collected daily, weighed, and a 5% daily aliquot was retained. The urine aliquots were pooled by steer and kept frozen at <−4°C. Fecal aliquots were dried at 60°C and pooled by steer. At the end of collection, each steer was removed from the crate and the crate was thoroughly scraped. Feces recovered from the crates were added to the fecal collection for that day.

Procedures for infusion of ^{15,15}N-urea are the same as those described in Exp.1, except urine was collected every 6 h starting at 32 h after initiation of isotope infusion. A separate aliquot of 125 mL was taken for ^{15}N analysis. The amount and pH of urine during these intervals was recorded, and the amount of the aliquot was included in the daily urine collection data.

After completion of the balance trial in period 1, steers returned to group housing. In period 2, a given steer remained on gamagrass or orchardgrass, but supplement treatment was switched.

Chemical Analysis

All feed, plant parts, feces, and orts samples were ground in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen. Samples were sealed in whirl-pack bags and stored at room temperature until analyzed. Urine samples were stored in 125-

mL plastic bottles and frozen at <−4°C until analyzed. Feed, feces, and orts were analyzed for DM, OM, and Kjeldahl N (AOAC, 1990). Duplicate samples of feed and orts were analyzed sequentially for NDF and ADF using the method of Van Soest et al. (1991) in a batch processor (Ankom Technology Corp., Fairport, NY). Whole hay and leaf, stem, heads, and other fractions were analyzed for NDF, ADF, and Kjeldahl N. In vitro NDF disappearance was determined by in vitro fermentation using the Ankom II Daisy batch fermenter (Ankom Technologies). Each fermenter received 2,000 mL of inoculate consisting of 1,600 mL of McDougall's buffer (Tilley and Terry, 1963) and 400 mL of strained rumen fluid. The rumen fluid came from a mature, fistulated steer fed a mixed alfalfa (*Medicago sativa* L.) and orchardgrass (*Dactylis glomerata* L.) hay. Samples (0.25 g) were weighed in quadruplicate in Ankom fiber bags (Ankom Technologies) and incubated for 0, 6, 12, 18, 24, 48, or 72 h. Fermentation was terminated by the NDF procedure in the Ankom 200 fiber analyzer. Sample fiber bags were then dried overnight at 60°C in a forced-air oven. Residue from the fiber bags was scraped into Kjeldahl tubes and analyzed for Kjeldahl N. Samples from 2 replicates were combined due to low amounts of residue, so the Kjeldahl N analyses were done in duplicate. The equation $y = ae^{-kt}$ was used to determine the rate of in vitro NDF disappearance and in vitro NDF-N disappearance, where y = NDF or NDF-N concentration, t = time of incubation in hours, k = rate/hour, and a = predicted concentration at time 0. Data from 0 and 6 h, which represented the lag phase of disappearance, were removed (Gill et al., 1969). The R^2 for the in vitro NDF disappearance or in vitro NDF-N disappearance regressions of whole plant, leaf, or stem components ranged from 0.85 to 0.86, and R^2 for in vitro NDF disappearance and in vitro NDF-N disappearance of supplement were 0.90 and 0.74, respectively.

Urea content of plasma and urine was determined using the diacetyl monoxime method of Marsh et al. (1957) adapted to a Technicon Auto Analyzer (Technicon Instrument Corp., Tarrytown, NY). Preparation and analysis of urine samples, and calculation of urea entry rate, urinary urea-N excretion, return of urea-N to the gut, and return of urea-N to the ornithine cycle have been described (Marini and Attene-Ramos, 2006; Huntington et al., 2007).

Statistical Analysis

Exp. 1. Data were statistically analyzed as a replicated Latin square using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC). The model included group, period, group × period, and treatment as fixed effects and steer within group as a random effect. Urine enrichments of urea-N were averaged within steer and period before statistical analysis. Results are reported as least-squares means. Data for 1 steer on the E+ diet, for the third period of the first square, were not used in the analysis, due to an elevated body temperature and change in intake during the final period. Statistical significance was declared at $P < 0.10$.

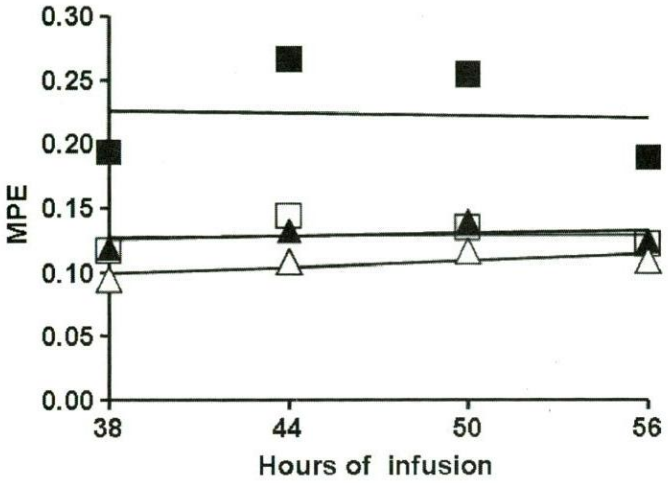

Figure 1. Molar percent excess (MPE) of ^{15,15}N urea in urine from steers fed gamagrass without supplement (□, SE = 0.031), gamagrass with supplement (■, SE = 0.059), orchardgrass without supplement (Δ, SE = 0.018), or orchardgrass with supplement (▲, SE = 0.017).

Because N intake differed among treatments (Matthews et al., 2005) and because of the relationship between N intake and urea entry rate (Archibeque et al., 2002; Marini and Van Amburgh, 2003; Marini et al., 2004; Wickersham et al., 2008a), dependent variables quantifying urea kinetics were adjusted by covariance analysis for N intake within steer and period.

Exp. 2. Due to errors in sampling, 3 of the possible 64 urine collections are missing, 1 for gamagrass, no supplement, 50 h of infusion; 1 for gamagrass with supplement, 44 h of infusion; and 1 for orchardgrass, no supplement, 50 h of infusion. Pooled regression within treatments (St. Pierre, 2001) showed no significant change ($P > 0.36$) with hours of ^{15,15}N-urea infusion for molar percent excess of $^{30}N_2$ (Figure 1), indicating that enrichment data from all time points could be used to calculate urea entry rate. However, both gamagrass treatments had slightly increasing molar percent excess of $^{29}N_2$ over time ($P < 0.06$, Figure 2), and therefore, slightly increasing ($P < 0.04$) return of urea-N to the ornithine cycle [molar percent excess of $^{29}N_2$/(molar percent excess of $^{29}N_2$ + $^{30}N_2$)]. Lobley et al. (2000) reported a similar response over a similar time frame for $^{29}N_2$ enrichment in sheep. Because of missing samples in our urine collections, because $^{29}N_2$ enrichment continued to increase throughout our collections, and because Lobley et al. (2000) recommended at least 48 h of isotope infusion before collection of urine for calculations, we decided to use urine collected from 50 to 56 h of isotope infusion to calculate urea kinetic parameters. We used the 5-d urinary urea excretion (Magee et al., 2005) as the best estimate for urinary urea excretion.

Data were statistically analyzed as a crossover 2 × 2 factorial design using the PROC MIXED procedure

Table 3. Nitrogen intake and urea-N kinetics (g of N/d) in Polled Hereford steers consuming endophyte-infected (E+), endophyte-free (E−), and nontoxic endophyte (NE) tall fescue in Exp. 2

Item	E+	E−	NE	SEM[1]	*P*-value
N intake	86	99	96	1.9	0.01
Urea entry (UER)[2]	74	127	116	14.3	0.10
Urine urea excretion (UUE)[2]	9.3	10.6	9.2	1.4	0.27
Returned to gut (GUR)[2]	64	117	108	14.7	0.09
Returned to ornithine cycle (ROC)[2]	10.1	24.6	23.2	4.4	0.10
UUE/UER	0.097	0.092	0.096	0.013	0.82
GUR/UER	0.903	0.908	0.904	0.013	0.82
ROC/GUR	0.192	0.194	0.197	0.012	0.90

[1]n = 8 for E− and NE, and n = 7 for E+; n = 7 for SEM.
[2]Adjusted by covariance analysis for N intake.

of SAS. The model included hay, supplement, hay × supplement, and period within hays as fixed effects and steer as a random effect. Hay was tested by the test term steers within hay, whereas the remaining sources of variation were tested against the residual mean squares. Statistical significance was declared at $P < 0.10$. As in Exp. 1, and for the same reasons, dependent variables quantifying urea kinetics were adjusted by covariance analysis for N intake within steer and period.

RESULTS AND DISCUSSION

Exp. 1

The hays in this experiment represent 3 main commercially available options for tall fescue as a forage source. Compared with E+, E− and NE had more protein and reduced concentrations of NDF and ADF ($P < 0.05$, Table 1). The E+ contained 0.120 mg/kg of ergovaline, and its true IVDMD (80.9%) was numerically less than the other hays (82.5 or 82.6%, SEM = 0.78; Matthews et al., 2005).

Compared with E− or NE, E+ had less ($P < 0.05$) DMI (5.00 vs. 5.24 or 5.19 kg/d), N intake (86 vs. 99 or 96 g/d), N digestibility (48.1 vs. 54.3 or 52.5%), and less N retained, expressed as grams per day (13.8 vs. 22.4 or 22.1), percentage of N intake (15.6 vs. 22.2 or 23), or percentage of N digested (32.7 vs. 41.4 or 43.6; Matthews et al., 2005). After adjustment for N intake by covariance, N digested was less ($P < 0.01$) for E+ than for E− or NE (43 vs. 52 or 50 g/d), but N retained did not differ among treatments ($P < 0.17$). Nonsignificant trends in N retained as a percentage of intake or as a percentage of N digested reported by Humphry et al. (2002) were similar to those reported for the hays in our study (Matthews et al., 2005); hay ergovaline concentrations were also similar between the studies.

After adjustment for differences in N intake, E+ had less ($P < 0.10$) urea entry rate, urea-N returned to the gut, and return of urea-N to the ornithine cycle than E− or NE (Table 3). However, urinary urea excretion, urea returned to the gut as a proportion of urea entry rate, and return of urea-N to the ornithine cycle as a percentage of urea returned to the gut were similar among treatments (Table 3), indicating metabolism of N through urea pathways was not a major determinant in the better efficiency of N capture by E− or NE than by E+ discussed in the previous paragraph. Urea recycling (urea returned to the gut) as a proportion of urea entry rate was greater than in other reports from steers fed endophyte-free fescue hay (Archibeque et al., 2002), but similar to proportions in steers fed hay containing 5% CP and supplemented with casein (Wickersham et al., 2008a).

These responses in N metabolism, plus less IVDMD and in vitro ADF digestibility for E+ (Matthews et al., 2005), are consistent with a reduction in ruminal fermentation when steers were fed E+ compared with the other hays. Others have reported minimal effects of endophyte infected tall fescue on ruminal concentrations of fermentation products or net absorption of nutrients by portal-drained viscera (Harmon et al., 1991), or in situ degradation of DM or fiber components (Humphry et al., 2002; Flores et al., 2007). However, Bush et al. (1976), Westendorf et al. (1993), and Humphry et al. (2002) reported decreased in vivo digestibilities of N, DM, NDF, or ADF in response to increased intake of alkaloids or endophyte-infected tall fescue.

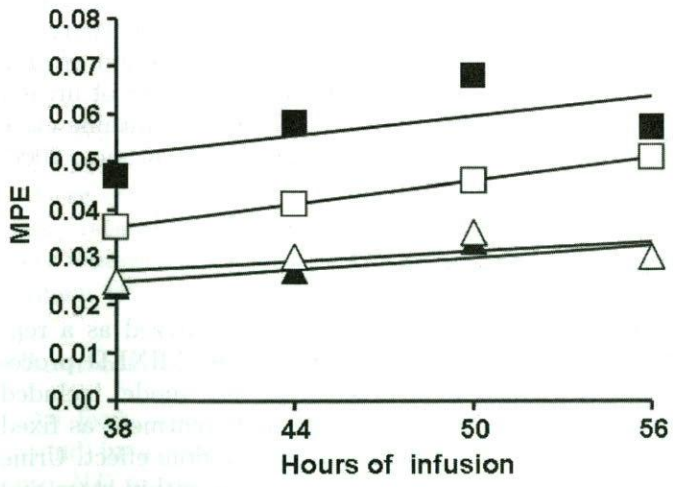

Figure 2. Molar percent excess (MPE) of ^{15,14}N urea in urine from steers fed gamagrass without supplement (□, SE = 0.012), gamagrass with supplement (■, SE = 0.008), orchardgrass without supplement (Δ, SE = 0.007), or orchardgrass with supplement (▲, SE = 0.008).

Table 4. Nitrogen intake and urea-N kinetics in steers fed gamagrass (GG) or orchardgrass (OG) hay with or without supplement in Exp. 2

	No supplement		Supplement			$P <$		
Item	GG	OG	GG	OG	SEM[1]	Hay (H)	Supplement (S)	H × S
N intake, g/d	74	89	90	106	4.6	0.06	0.01	0.93
Urea entry (UER),[2,3] g of N/d	78	84	50	69	11	0.32	0.06	0.19
Urine urea excretion (UUE),[3] g of N/d	11	28	5	16	2.4	0.01	0.01	0.01
Recycled to gut (GUR),[2,3] g of N/d	68	56	45	53	11	0.85	0.24	0.16
Return to ornithine cycle (ROC),[3] g of N/d	16.7	10.0	8.5	9.8	3.7	0.35	0.45	0.06
UUE/UER	0.101	0.338	0.104	0.285	0.030	0.01	0.43	0.38
GUR/UER	0.899	0.662	0.896	0.715	0.030	0.01	0.43	0.38
ROC/GUR	0.269	0.147	0.211	0.139	0.018	0.01	0.09	0.17

[1]n = 4 for each mean.
[2]Calculated from urine collected from 50 to 56 h after start of infusion.
[3]Adjusted by covariance analysis for differences in N intake within steer and period.

Exp. 2

Compared with orchardgrass, gamagrass contained less CP, NDF ($P < 0.01$), and ADF ($P < 0.10$), and more OM ($P < 0.01$, Table 2). By design, the composited sample of supplement had greater in vitro NDF disappearance (4.42%/h) than the composited sample of gamagrass (1.06%/h) or orchardgrass (1.2%/h), and less in vitro NDF-N disappearance (1.85%/h) than the composited sample of gamagrass (2.56%/h) or orchardgrass (2.32%/h). Therefore, supplementation of both hays increased readily fermentable, nonstructural carbohydrates (corn starch), and ostensibly increased ruminal cell wall digestibility and decreased ruminal NDF-N digestibility of the total diet.

The hays used in this experiment were chosen because we expected differences in leaf to stem ratios (Cherney et al., 1990) and protein fractions (Sniffen et al., 1992) between these warm- and cool-season grasses. These compositional changes cause distinct ruminal disappearance rates, which in turn affect N metabolism (Redfearn et al., 1995). We expected the gamagrass to have a greater leaf to stem ratio and different leaf characteristics than orchardgrass. However, the hand separations and in vitro incubations indicated that the ratios and fermentation rates did not differ as expected. The composited sample of orchardgrass had a greater leaf to stem ratio than gamagrass (4.2:1 vs. 2.3:1) and greater whole plant in vitro NDF disappearance (1.20 vs. 1.06%/h). The leaf in vitro NDF disappearance (1.21%/h) and in vitro NDF-N disappearance (2.15%/h) was almost twice as fast as the stem in vitro NDF disappearance (0.72%/h) and in vitro NDF-N disappearance (1.2%/h) for both gamagrass and orchardgrass. These results are in agreement with the concept that leaf is composed of less fiber and is more digestible than stem and that the leaf to stem ratio of the whole plant will have a direct effect on the energy and protein release in the rumen. The composited sample of orchardgrass had a greater concentration of CP in leaf than gamagrass (Table 2), but in vitro NDF-N disappearance was greater for the composited sample of gamagrass (2.66%/h) than orchardgrass (1.63%/h). Such differences are consistent with anatomical differences between C3 (orchardgrass) and C4 (gamagrass) plants because C3 grasses have a greater proportion of the leaf cross-sectional area that is rapidly degraded in the rumen (56 to 66% of tissue) than C4 grasses (27 to 55% of tissue). Correspondingly, slowly degradable tissue is 22 to 31% of cross-sectional area in C3 grasses and 36 to 56% of cross-sectional area in C4 grasses (Akin et al., 1973; Akin and Burdick, 1975).

Compared with steers fed orchardgrass, steers fed gamagrass had less ($P < 0.06$) N intake (87 vs. 92 g/d), less ($P < 0.05$) urine N output (35 vs. 38 g/d), less ($P < 0.01$) plasma urea-N concentrations (2.6 vs. 7.1 m*M*), and less ($P < 0.06$) N digested (47 vs. 59 g/d, Magee et al., 2005). After adjustment by covariance analysis for differences in N intake, forage species did not affect ($P > 0.32$) urea entry rate, urea returned to the gut, or return of urea-N to the ornithine cycle (Table 4). Urinary urea-N excretion adjusted for N intake was greater ($P < 0.01$) for steers fed orchardgrass, but urea returned to the gut as a proportion of urea entry rate and urea-N returned to the ornithine cycle as a proportion of urea-N recycled to the gut were greater ($P < 0.01$) for steers fed gamagrass. Forage species did not affect fecal N output or N retained (27 g/d; Magee et al., 2005). Compared with steers fed orchardgrass, steers fed gamagrass had greater ($P < 0.05$) N retention as a percentage of N intake (36 vs. 25), increased ($P < 0.01$) N retained as a percentage of N digested (63 vs. 41), but similar N digested as a percentage of N intake (57 vs. 60; Magee et al., 2005). Therefore, N balance and urea kinetics indicate more efficient use of N by steers fed gamagrass.

Supplement increased ($P < 0.01$) N intake from 81 to 98 g/d, N digested from 47 to 59 g/d, and fecal N from 33 to 40 g/d (Magee et al., 2005). After adjustment by covariance analysis for differences in N intake, supplement decreased ($P < 0.06$) urea entry rate (Table 4). Plasma urea-N concentrations also increased from 5.4 to 4.3 m*M* ($P < 0.01$, Magee et al., 2005). Supplement increased ($P < 0.01$) N retained from 22 to 33 g/d, N

retained as a percentage of N intake from 27 to 34% ($P < 0.05$), and N retained as a percentage of N digested from 46 to 58% ($P < 0.01$; Magee et al., 2005). Supplement also decreased ($P < 0.09$) return of urea-N to the ornithine cycle as a proportion of urea-N recycled to the gut (Table 4); ostensibly, a greater proportion of recycled urea-N was incorporated into microbial protein.

There were hay source × supplement interactions (Magee et al., 2005; Table 4) for urine urea-N excretion adjusted or not adjusted for N intake ($P < 0.04$), urine urea as a percentage of total urinary N ($P < 0.05$), N retained as a percentage of N digested ($P < 0.10$), return of urea-N to the ornithine cycle as a proportion of urea returned to the gut ($P < 0.09$), return of urea N to the ornithine cycle ($P < 0.06$), and plasma urea-N concentrations ($P < 0.03$). There was a trend ($P < 0.15$) for a hay source × supplement interaction for N retained as a percentage of N intake. The interactions were due to a greater response to supplement for steers fed orchardgrass than for those fed gamagrass. As previously discussed, orchardgrass had greater leaf:stem ratio, greater CP content in the leaf, and greater in vitro NDF disappearance than gamagrass, but it also had greater NDF and ADF concentration and less in vitro NDF-N disappearance than gamagrass. Apparently, the increased supply of fermentable carbohydrate, and not slower in vitro NDF-N disappearance in the supplement allowed orchardgrass to respond more than gamagrass to improve efficiency of N capture by the steers. Recent studies with sheep (Kiran and Mutsvangwa, 2007; Sunny et al., 2007), steers (Wickersham et al., 2008b), heifers (Marini and Van Amburgh, 2003), and dairy cows (Gozho et al., 2008) have shown a positive relationship between fermentable carbohydrate and capture of fermentable N sources (including recycled urea-N) as microbial protein.

Return of Urea-N to the Ornithine Cycle

Data from the current experiments were combined with unpublished data from other experiments with steers fed high- or all-forage diets (Archibeque et al., 2001, 2002; Huntington et al., 2007) to assess relationships between the proportion of urea-N returning to the ornithine cycle [molar percent excess of $^{29}N_2$/(molar percent excess of $^{29}N_2$ + $^{30}N_2$)] and urea-N entry rate or recycling of urea-N to the gut. For 112 steer means within treatments and experiments, the range and mean ± SD proportion of urea-N returning to the ornithine cycle was 0.120 to 0.317 and 0.207 ± 0.043, respectively. There was no significant correlation between the proportion of urea-N returning to the ornithine cycle and urea-N entry or recycling of urea-N to the gut for the 112 steer means ($P > 0.14$), or for treatment means (n = 18) among the experiments ($P > 0.24$). Lack of correlation indicates the proportion of urea-N returning to the ornithine cycle is affected by factors other than urea supply to the bloodstream or endogenous urea recycling to the gut.

Conclusions

Steers in both studies recycled to the gut 80% or more of urea produced, which reflects the ability of steers to retrieve urea-N when N intake is low relative to requirements. After adjustment for differences among treatment N supply, results of both experiments support the concept of improved N use in response to increased carbohydrate fermentability in the rumen, due either to inherent differences in forage fiber or to supplementation with readily fermentable carbohydrate (starch or fiber). Closer coordination of ruminal fermentation of carbohydrate and N sources provided greater and more efficient capture of dietary N as tissue protein in forage-fed steers. Acceptably stable enrichment of ^{15}N-urea in urine was obtained after 50 h of intrajugular infusion of ^{15,15}N-urea, indicating that valid data on urea metabolism can be obtained from steers fed forages twice daily as well as those fed more frequently. Waiting for at least 48 h after the start of infusion to collect enriched urine samples, as previously suggested by Lobley et al. (2000), allows more time for equilibration of the ^{15,14}urea-N pool resulting from return of urea-N from the gut to the ornithine cycle and is recommended in future studies with meal-fed ruminants.

LITERATURE CITED

Akin, D. E., H. E. Amos, F. E. Barton II, and D. Burdick. 1973. Rumen microbial degradation of grass tissue revealed by scanning electron microscopy. Agron. J. 65:825–828.

Akin, D. E., and D. Burdick. 1975. Percentage of tissue types in tropical and temperate grass leaf blades and degradation of tissue by microorganisms. Crop Sci. 15:661–668.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Archibeque, S. L., J. C. Burns, and G. B. Huntington. 2001. Urea flux in beef steers: Effects of forage species and nitrogen fertilization. J. Anim. Sci. 79:1937–1943.

Archibeque, S. L., J. C. Burns, and G. B. Huntington. 2002. Nitrogen metabolism of beef steers fed endophyte-free tall fescue hay: Effects of ruminally protected methionine supplementation. J. Anim. Sci. 80:1344–1351.

Bush, L. P., H. Burton, and J. A. Boling. 1976. Activity of tall fescue alkaloids and analogues in in vitro rumen fermentation. J. Agric. Food Chem. 24:869–872.

Cherney, D. J. R., D. R. Mertens, and J. E. Moore. 1990. Intake and digestibility of wethers as influenced by forage morphology at three levels of forage offering. J. Anim. Sci. 68:4387–4399.

El-Kadi, S. W., R. L. Baldwin VI, N. E. Sunny, S. L. Owens, and B. J. Bequette. 2006. Intestinal protein supply alters amino acids, but not glucose, metabolism by the sheep gastrointestinal tract. J. Nutr. 136:1261–1269.

Flores, R., W. K. Coblentz, R. K. Ogden, K. P. Coffey, M. L. Looper, C. P. West, and C. R. Rosenkrans Jr. 2007. Effects of fescue type and sampling date on the ruminal disappearance kinetics of autumn-stockpiled tall fescue. J. Dairy Sci. 90:2883–2896.

Gill, S. S., H. R. Conrad, and J. W. Hibbs. 1969. Relative rate of in vitro cellulose disappearance as a possible estimator of digestible dry matter intake. J. Dairy Sci. 52:1687–1690.

Gozho, G. N., M. R. Hobin, and T. Mutsvangwa. 2008. Interactions between barley grain processing and source of supplemental dietary fat on nitrogen metabolism and urea-nitrogen recycling in dairy cows. J. Dairy Sci. 91:247–259.

Harmon, D. L., K. L. Gross, K. K. Kreikemeier, K. P. Coffey, T. B. Avery, and J. Klindt. 1991. Effects of feeding endophyte-infected fescue hay on portal and hepatic nutrient flux in steers. J. Anim. Sci. 69:1223–1231.

Humphry, J. B., K. P. Coffey, J. L. Moyert, F. K. Brazle, and L. W. Lomas. 2002. Intake, digestion, and digestive characteristics of *Neotyphodium coenophialum*-infected and uninfected fescue by heifers offered hay diets supplemented with *Aspergillus oryzae* fermentation extract or laidlomycin propionate. J. Anim. Sci. 80:225–234.

Huntington, G. B., and J. C. Burns. 2008. The interaction of harvesting time of day of switchgrass hay and ruminal degradability of supplemental protein offered to beef steers. J. Anim. Sci. 86:159–166.

Huntington, G. B., J. C. Burns, and S. A. Archibeque. 2007. Urea metabolism in beef steers grazing Bermudagrass, Caucasian Bluestem, or gamagrass pastures varying in plant morphology, protein content, and protein composition. J. Anim. Sci. 85:1997–2004.

Kiran, D., and T. Mutsvangwa. 2007. Effects of barley grain processing and dietary ruminally degradable protein on urea nitrogen recycling and nitrogen metabolism in growing lambs. J. Anim. Sci. 85:3391–3399.

Lobley, G. E., D. M. Bremner, and G. Zuur. 2000. Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive [$^{15}N^{15}N$]urea kinetics. Br. J. Nutr. 84:459–468.

Magee, K. J., M. H. Poore, J. C. Burns, and G. B. Huntington. 2005. Nitrogen metabolism in beef steers fed gamagrass or orchardgrass hay with or without a supplement. Can. J. Anim. Sci. 85:107–109.

Marini, J. C., and M. S. Attene-Ramos. 2006. An improved analytical method for the determination of urea nitrogen isotopomers in biological samples utilizing continuous flow isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom. 20:3736–3740.

Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. J. Anim. Sci. 82:1157–1164.

Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545–552.

Marsh, W. H., B. Fingerhut, and E. Kirsch. 1957. Determination of urea N with the diacetyl method and an automatic dialyzing apparatus. Am. J. Clin. Pathol. 8:681–688.

Matthews, A. K., M. H. Poore, G. B. Huntington, and J. T. Green. 2005. Intake, digestion, and N metabolism in steers fed endophyte-free, ergot alkaloid-producing endophyte-infected, or nonergot alkaloid-producing endophyte-infected fescue hay. J. Anim. Sci. 83:1179–1185.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Redfearn, D. D., L. E. Moser, S. S. Waller, and T. J. Klopfenstein. 1995. Ruminal degradation of switchgrass, big bluestem, and smooth bromegrass leaf proteins. J. Anim. Sci. 73:598–605.

Reynolds, C. K., and N. B. Kristensen. 2008. Nitrogen recycling through the gut and nitrogen economy of ruminants: An asynchronous symbiosis. J. Anim. Sci. 86:E293–E305.

Sniffen, C. J., J. D. O'Conner, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70:3562–3577.

St-Pierre, N. R. 2001. Integrating quantitative finding from multiple studies using mixed model methodology. J. Dairy Sci. 84:741–755.

Sunny, N. E., S. L. Owens, R. L. Baldwin VI, S. W. El-Kadi, R. A. Kohn, and B. J. Bequette. 2007. Salvage of blood urea nitrogen in sheep is highly dependent on plasma urea concentration and the efficiency of capture within the digestive tract. J. Anim. Sci. 85:1006–1013.

Tilley, J. M., and R. A. Terry. 1963. A two-stage technique for in vitro digestion of forage crops. J. Br. Grassl. Soc. 18:104–111.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Westendorf, M. L., G. E. Mitchell Jr., R. E. Tucker, L. P. Bush, R. J. Petroski, and R. G. Powell. 1993. In vitro and in vivo ruminal and physiological responses to endophyte-infected tall fescue. J. Dairy Sci. 76:555–563.

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and D. P. Gnad. 2008a. Effect of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3079–3088.

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, E. E. Wickersham, and E. S. Moore. 2008b. Effect of frequency and amount of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. J. Anim. Sci. 86:3089–3099.